We claim:

1. A recombinant vector comprising foreign nucleic acid sequences encoding multiple costimulatory molecules or functional portions thereof.

2. The recombinant vector according to claim 1 wherein the nucleic acid sequences encode at least three costimulatory molecules.

3. The recombinant vector according to claim 1 wherein the nucleic acid sequence encoding each costimulatory molecule is derived from a mammalian source.

4. The recombinant vector according to claim 1 wherein the multiple costimulatory molecules are selected from the group consisting of B7-1, B7-2, ICAM-1, LFA-3, 4-1BBL, CD59, CD40, CD70, OX-40L, VCAM-1 and mammalian homologs thereof.

5. The recombinant vector according to claim 5 wherein the multiple costimulatory molecules are B7-1, ICAM-1 and LFA-3.

6. The recombinant vector according to claim 1 further comprising a foreign nucleic acid sequence encoding at least one cytokine, chemokine, Flt-3L, or combination thereof.

7. The recombinant vector according to claim 1 further comprising a multiplicity of promoters.

8. The recombinant vector according to claim 7 wherein the promoters are derived from a eukaryotic source, prokaryotic source, or viral source.

9. The recombinant vector according to claim 7 wherein the promoters are selected from the group consisting of an SV40 early promoter, RSV promoter, adenovirus major late promoter, human CMV immediate early I promoter, poxvirus promoter, 30K promoter, I3 promoter, sE/L promoter, 7.5K promoter, 40K promoter, and C1 promoter.

10. The recombinant vector according to claim 1 wherein the recombinant vector is selected from the group consisting of a bacteria, virus, and nucleic acid-based vector.

11. The recombinant vector according to claim 1 wherein the recombinant vector is selected from the group consisting of poxvirus, adenovirus, Herpes virus, alphavirus, retrovirus, picornavirus, and iridovirus.

12. The recombinant vector according to claim 11 wherein the recombinant vector is a recombinant poxvirus.

13. The recombinant vector according to claim 12 wherein the recombinant poxvirus is a replicating virus or a non-replicating virus.

14. The recombinant vector according to claim 12 wherein the recombinant poxvirus is orthopox, avipox, capripox or suipox.

15. The recombinant vector according to claim 14 wherein the avipox is fowlpox, canary pox or derivatives thereof.

16. The recombinant vector according to claim 14 wherein the orthopox is vaccinia, vaccinia-Copenhagen strain, vaccinia-Wyeth strain, NYVAC, vaccinia-MVA strain, raccoon pox or rabbit pox.

17. The recombinant vector according to claim 1 further comprising a foreign nucleic acid sequence encoding at least one target antigen or immunological epitope thereof.

18. The recombinant vector according to claim 17 wherein the target antigen has an amino acid sequence selected from the group consisting of SEQ ID NO: 2 through SEQ ID NO: 40.

19. The recombinant vector according to claim 17 wherein the target antigen is selected from the group consisting of a tumor specific antigen, tumor associated antigen, tissue-specific antigen, bacterial

antigen, viral antigen, yeast antigen, fungal antigen, protozoan antigen, and parasite antigen and mitogen.

20. The recombinant vector according to claim 18 wherein the bacterial antigen is derived from a bacterium selected from the group consisting of Chlamydia, Mycobacteria. Legionella, Meningiococcus, Group A Streptococcus, *Hemophilus influenzae*, Salmonella, and Listeria.

21. The recombinant vector according to claim 18 wherein the viral antigen is derived from a virus selected from the group consisting of Lentivirus, Herpes virus, Hepatitis virus, Orthomyxovirus and Papillomavirus.

22. The recombinant vector according to claim 21 wherein the Lentivirus is HIV-1 or HIV-2.

23. The recombinant vector according to claim 21 wherein the Herpes virus is HSV or CMV.

24. The recombinant vector according to claim 21 wherein the Hepatitis virus is Hepatitis A, Hepatitis B, Hepatitis C, Hepatitis D or Hepatitis E.

25. The recombinant vector according to claim 21 wherein the orthomyxovirus is influenza virus.

26. The recombinant vector according to claim 18 wherein the tumor associated antigen, tumor specific antigen or tissue-specific antigen is selected from the group consisting of CEA, MART-1, MAGE-1, MAGE-3, GP-100, MUC-1, MUC-2, pointed mutated ras oncogene, normal or point mutated p53, overexpressed p53, CA-125, PSA, C-erb/B2, BRCA I, BRCA II, PSMA, tyrosinase, TRP-1, TRP-2, NY-ESO-1, TAG72, KSA, HER-2/neu, bcr-abl, pax3-fkhr, ews-fli-1, modified TAAs, splice variants of TAAs, functional epitopes and epitope agonists thereof.

27. The recombinant vector according to claim 26 wherein the antigen is CEA (6D) having an amino acid aspartic acid at amino acid position 576.

28. The recombinant vector according to claim 26 wherein the antigen is PSA and PSMA.

29. The recombinant vector according to claim 26 wherein the antigen is MUC-1 encoded by a truncated MUC-1 gene consisting of a signal sequence, ten copies of a tandem repeat sequence, and a 3' coding sequence.

30. The recombinant vector according to claim 18 wherein the yeast or fungal antigen is derived from a yeast or fungus selected from the group consisting of Aspergillus, Nocardia, Histoplasmosis, Candida, and Cryptosporidia.

31. The recombinant vector according to claim 18 wherein the parasitic antigen is derived from a *Plasmodium species*, *Toxoplasma gondii*, *Pneumocystis carinii*, *Trypasosoma species*, or *Leishmania species*.

32. The recombinant vector according to claim 1 wherein the vector further comprises a selectable marker.

33. The recombinant vector according to claim 32 wherein the selectable marker is selected from the group consisting of *lacZ* gene, thymidine kinase, gpt, GUS and a vaccinia K1L host range gene.

34. A pharmaceutical composition comprising at least one recombinant vector according to any of claims 1-33 and a pharmaceutically acceptable carrier.

35. A pharmaceutical composition comprising at least one recombinant vector according to any of claims 1-16, a second recombinant vector comprising at least one nucleic acid sequence encoding at least one

target antigen or immunological epitope thereof and a pharmaceutically acceptable carrier.

36. A pharmaceutical composition according to claim 34 or 35 further comprising a cytokine, chemokine or Flt-3L.

37. A host cell infected, transfected or induced with the recombinant vector according to any of claims 1-33.

38. The host cell infected, transfected or induced with the recombinant vector according to claims 1-16 and infected, transfected or induced with a second recombinant vector comprising at least one foreign nucleic acid sequence encoding at least one target antigen or immunological epitope thereof.

39. The host cell according to claim 38 wherein the host cell is an antigen presenting cell or precursor thereof, a premalignant cell, a hyperplastic cell or tumor cell.

40. The host cell according to claim 39 wherein the antigen presenting cell is a dendritic cell or precursor thereof, a monocyte, macrophage, B-cell, fibroblast or muscle cell.

41. The host cell according to claim 39 wherein the antigen presenting cell is derived from bone marrow, spleen, skin, peripheral blood, tumor, lymph node, or muscle.

42. The host cell according to claim 38 wherein the host cell is an antigen presenting cell or precursor thereof, a premalignant cell, a hyperplastic cell or tumor cell.

43. The host cell according to claim 42 wherein the antigen presenting cell is a dendritic cell, precursor or derivative thereof, a monocyte, macrophage, B-cell, fibroblast or muscle cell.

44. The host cell according to claim 43 wherein the derivative is a TNF α-treated dendritic cell, a CD40-treated dendritic cell, or a subpopulation of adherent cells.

45. A dendritic cell or precursor thereof comprising a foreign nucleic acid sequence encoding multiple costimulatory molecules.

46. A tumor cell or precursor thereof comprising a foreign nucleic acid sequence encoding multiple costimulatory molecules.

47. The cell according to claims 45 or 46 wherein the cell comprises a foreign nucleic acid sequence encoding at least three costimulatory molecules.

48. The cell according to claims 45 or 46 wherein the multiple costimulatory molecules are selected from the group consisting of B7-1, B7-2, ICAM-1, LFA-3, 4-1BBL, CD59, CD40, CD70, OX-40L, VCAM-1, mammalian homologs thereof and combinations thereof.

49. The cells according to claims 45 or 46 wherein the multiple costimulatory molecules are at least B7-1, ICAM-1 and LFA-3.

50. The cells according to claims 45 or 46 further comprising a foreign nucleic acid sequence encoding at least one target antigen or immunological epitope thereof.

51. The cells according to claim 50 wherein the foreign nucleic acid sequence encoding at least one target antigen or immunological epitope thereof is provided by a recombinant vector, RNA or DNA from a tumor cell lysate, or by fusion with a tumor cell comprising said sequence.

52. The cells according to claim 50 wherein the target antigen or immunological epitope thereof is selected from the group consisting of a tumor specific antigen, tumor associated antigen, tissue-specific

antigen, bacterial antigen, viral antigen, yeast antigen, fungal antigen, protozoan antigen, parasite antigen and mitogen.

53. A pharmaceutical composition comprising the cells according to claims 45-52 and optionally an exogenous source of target antigen or immunological epitope thereof.

54. A recombinant poxvirus having integrated into a viral genome foreign DNA encoding multiple costimulatory molecules produced by a process comprising: allowing a plasmid vector comprising the foreign DNA encoding multiple costimulatory molecules to undergo recombination with a parental poxvirus genome to produce a recombinant poxvirus having inserted into its genome the foreign DNA.

55. A recombinant poxvirus having integrated into a viral genome foreign DNA encoding LFA-3, ICAM-1 and at least one B7 molecule produced by a process comprising: allowing a plasmid vector comprising the foreign DNA encoding LFA-3, ICAM-1 and at least one B7 molecule to undergo recombination with a parental poxvirus genome to produce a recombinant poxvirus having inserted into its genome the foreign DNA.

56. The recombinant poxvirus according to claim 54 or 55, wherein the genome further comprises a multiplicity of poxvirus promoters capable of controlling expression of the foreign DNA.

57. The recombinant poxvirus according to claims 54 or 55 further comprising a foreign gene encoding at least one target antigen or immunological epitope thereof.

58. A pharmaceutical composition comprising a recombinant poxvirus according to any of claims 54-57 and a pharmaceutically acceptable carrier.

59. A pharmaceutical composition comprising a recombinant poxvirus according to any of claims 54-57 further comprising a second

recombinant poxvirus comprising at least one foreign nucleic acid sequence encoding at least one target antigen or immunological epitope thereof.

60. A host cell infected with the recombinant poxvirus according to claims 54-57.

61. The host cell according to claim 60, wherein the cell is a progenitor antigen presenting cell, an antigen presenting cell or an engineered antigen presenting cell.

62. The host cell according to claim 61, wherein the cell is a progenitor dendritic cell, dendritic cell, monocyte, macrophage, B-cell, fibroblast or muscle cell.

63. The host cell according to claim 60, wherein the cell is a hyperplastic cell, premalignant cell or a tumor cell.

64. A plasmid vector comprising nucleic acid sequences encoding multiple costimulatory molecules or functional portions thereof.

65. The plasmid vector according to claim 64 further comprising a gene encoding a selectable marker.

66. The plasmid vector according to claim 64 wherein the costimulatory molecules are human derived, non-human primate derived or murine derived.

67. The plasmid vector according to claim 64 further comprising a foreign nucleic acid sequence encoding at least one target antigen or immunological epitope thereof.

68. The plasmid vector according to claim 67 wherein the target antigen is selected from the group consisting of a tumor specific antigen, tumor associated antigen, tissue specific antigen, bacterial antigen, viral

antigen, yeast antigen, fungal antigen, protozoan antigen, parasite antigen, and mitogen.

69. The plasmid vector according to claim 68 wherein the target antigen is carcinoembryonic antigen (CEA) or immunological epitope thereof.

70. A plasmid vector for recombination with a poxvirus designed to produce a recombinant poxvirus capable of expressing foreign nucleic acid sequences encoding three costimulatory molecules, LFA-3, ICAM-1 and at least one B7 molecule which comprises (a) a multiplicity of poxviral promoters, (b) the foreign nucleic acid sequences encoding LFA-3, ICAM-1 and at least one B7 molecule, (c) DNA sequences flanking the construct of elements (a) and (b), the flanking sequences of both the 5' and 3' ends being homologous to a region of a parental poxvirus genome where elements (a) and (b) are to be inserted.

71. The plasmid vector according to claim 64 designated pT5064 deposited with the ATCC under Accession No. 203482

72. The plasmid vector according to claim 67, designated pT5049 deposited with the ATCC under Accession No. 203481.

73. A kit for use in making a recombinant poxvirus comprising a plasmid vector according to any of claims 64-72 and optionally a parental poxvirus.

74. A method of making a recombinant poxvirus comprising allowing the plasmid vector according to claim 64-72 to undergo recombination with a parental poxvirus genome to produce a recombinant poxvirus having inserted into its genome the foreign DNA and a multiplicity of poxvirus promoters capable of controlling the expression of the foreign DNA.

75. A method of enhancing an immune response in an individual comprising administration of a recombinant vector according to claims 1-33 in an amount sufficient to enhance the immune response.

76. The method according to claim 75 wherein a route of administration is intravenous, subcutaneous, intralymphatic, intratumoral, intradermal, intramuscular, intraperitoneal, intrarectal, intravaginal, intranasal, oral, via bladder instillation, or via scarification.

77. The method according to claim 75 wherein the enhanced immune response is a cell mediated or humoral response.

78. The method according to claim 75, wherein the enhancement is of $CD4^+$ T cell proliferation, $CD8^+$ T cell proliferation, or combination thereof.

79. The method according to claim 75, wherein the enhancement is of $CD4^+$ T cell function, $CD8^+$ T cell function or combination thereof.

80. The method according to claim 75, wherein the enhancement is in IL-2 production, IFN-γ production or combination thereof.

81. The method according to claim 75, wherein the enhancement is of antigen presenting cell proliferation, function or combination thereof.

82. A method of enhancing an antigen-specific T-cell response in an individual to a target antigen or immunological epitope thereof comprising administering a recombinant poxvirus comprising a foreign nucleic acid sequence encoding at least one B7 molecule, a foreign nucleic acid sequence encoding ICAM-1, and a nucleic acid sequence encoding LFA-3, and optionally a nucleic acid sequence encoding a target antigen or immunological epitope thereof, each nucleic acid sequence expressed in an infected cell in the individual in an amount effective to enhance at least one T-cell response, wherein the enhancement is greater than the enhancement obtained using a single costimulatory molecule or two costimulatory molecules.

83. The method according to claim 82, wherein the enhancement is of $CD4^+$ T cell proliferation, $CD8^+$ T cell proliferation, or combination thereof.

84. The method according to claim 82, wherein the enhancement is in IL-2 production, IFN-γ production or combination thereof.

85. The method according to claim 82, wherein the enhancement is of antigen-specific cytotoxicity.

86. The method according to claim 82 wherein the infected cell is an antigen presenting cell.

87. The method according to claim 86, wherein the cell is a dendritic cell, precursor thereof, monocyte, macrophage, B-cell fibroblast or muscle cell.

88. The method according to claim 82, wherein the infected cell is a tumor cell or precursor thereof.

89. A method of treatment or prevention of disease in an individual comprising:

(a) activating a T lymphocyte by exposing the T lymphocyte in vitro to a cell according to claim 37 alone or in combination with a target antigen or immunological epitope thereof;

(b) administering the activated T lymphocyte to an individual alone, or in combination with the target antigen in an amount sufficient to enhance an immune response.

90. The method according to claim 89 wherein the T lymphocytes are autologous with the individual.

91. The method according to claim 89, further comprising the administration of a cytokine, chemokine, flt-31 or combination thereof.

92. The method according to claim 89 wherein the immune response is against the target antigen selected from the group consisting of a tumor specific antigen, tumor associated antigen, tissue-specific antigen, bacterial antigen, viral antigen, yeast antigen, fungal antigen, protozoan antigen, and parasite antigen.

93. The method according to claim 89 wherein the immune response prevents or treats a disease caused by a cell or organism selected from the group consisting of viruses, bacteria, protozoans, parasites, premalignant cells and tumor cells.

94. A method of enhancing an immune response in an individual comprising administration of a cell according to any of claims 45-52 in an amount effective to enhance an immune response.

95. A method of enhancing an immune response in an individual comprising administration of a tumor cell, or precursors thereof according to claim 46 in an amount effective to enhance an immune response.

96. The method according to claims 94 or 95 wherein the cells are autologous, syngeneic or allogeneic with the individual.

97. The method according to claims 94 or 95 wherein the cells have been pulsed with a target antigen or epitope thereof.

98. The method according to claims 94 or 95 further comprising the administration of a target cell, target antigen or immunological epitope thereof.

99. The method according to claim 94 further comprising the administration of activated, target antigen specific lymphocytes.

100. A method for making a progenitor dendritic cell or dendritic cell that overexpresses multiple costimulatory molecules, said method comprising:

(a) providing the cell with a recombinant vector comprising foreign genes encoding multiple costimulatory molecules for a period of time sufficient to cause overexpression of the multiple costimulatory molecules by the cells.

101. The method according to claim 100 wherein the cells are isolated from bone marrow or peripheral blood mononuclear cells.

102. The method according to claim 100 wherein the recombinant vector further comprises a foreign gene encoding at least one target antigen or immunological epitope thereof.

103. The method according to claim 100 further comprising (b) providing the cell with a second recombinant vector comprising a foreign gene encoding at least one target antigen or immunological epitope thereof.

104. An *in vitro* method of assessing efficacy of a vaccine against a target antigen comprising:

(a) obtaining lymphocytes from an individual previously vaccinated with a target antigen or epitope thereof,

(b) determining the number and function of target-antigen specific lymphocytes in the presence of antigen presenting cells according to claim 39, an increase in number, function or combination thereof of target-antigen specific lymphocytes being indicative of efficacy of the vaccine.

105. A method of screening for novel immunogenic peptides from a multiplicity of peptides comprising:

(a) pulsing antigen presenting cells infected with a recombinant vector encoding multiple costimulatory molecules with a multiplicity of peptides to form peptide-pulsed antigen presenting cells;
(b) measuring lymphoid immunoreactivity in the presence of the peptide-pulsed antigen presenting cells, wherein enhanced immunoreactivity is indicative of an immunogenic peptide on the peptide-pulsed antigen presenting cell.

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106. The method according to claim 105, wherein the source of the multiplicity of peptides is a combinatorial peptide library.

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